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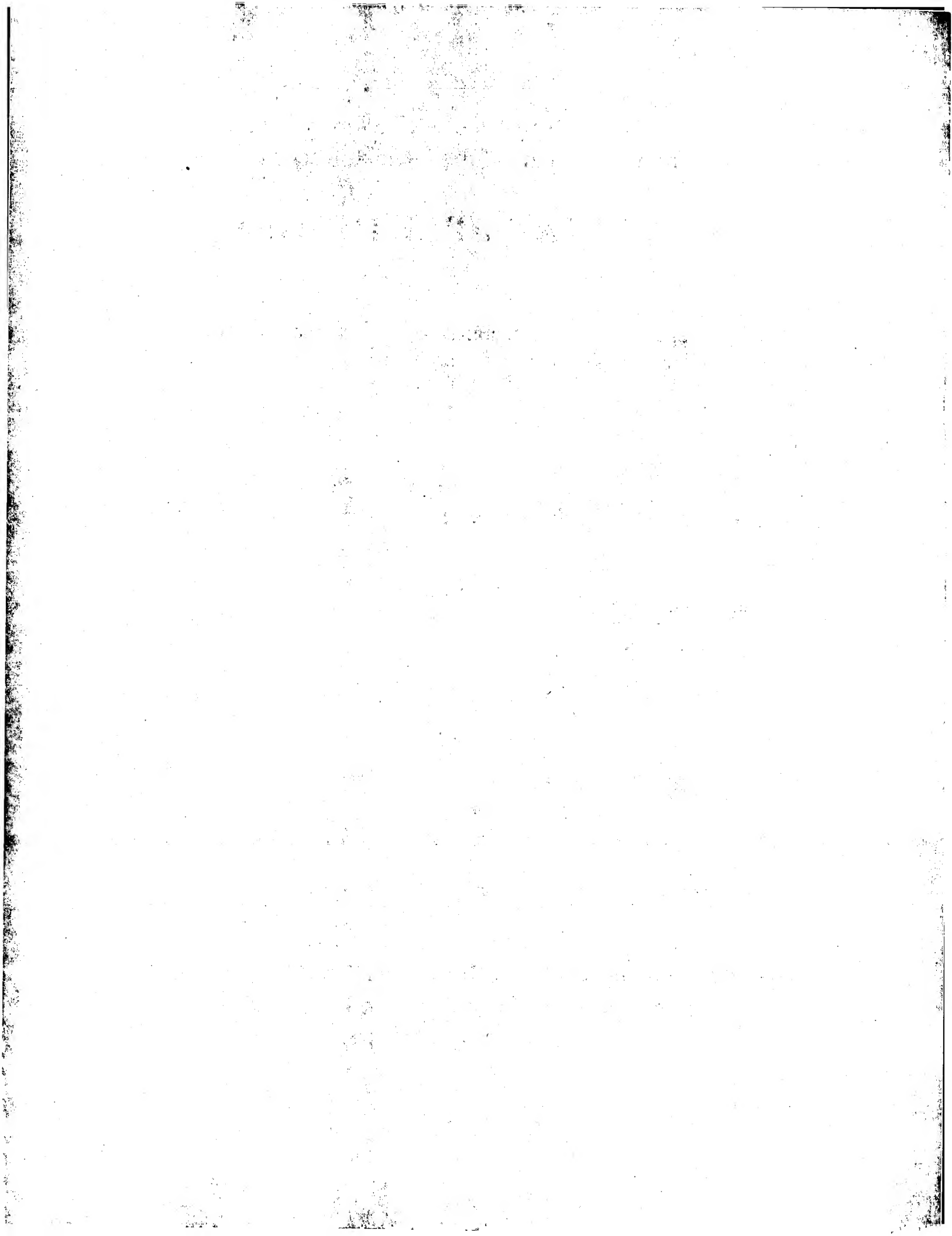
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(21) International Application Number: PCT/US91/01545 (22) International Filing Date: 6 March 1991 (06.03.91) (30) Priority data: 489,815 6 March 1990 (06.03.90) US (71) Applicant: CURATIVE TECHNOLOGIES, INC. [US/ US]; 14 Research Way, Setauket, NY 11733 (US). (72) Inventor: NEWMAN, Dawn, D. ; 67 Inlet View Path, East Moriches, NY 11940 (US). (74) Agents: POPOVICH, Thomas, E. et al.; Dorsey & Whit- ney, 2200 First Bank Place East, Minneapolis, MN 55402 (US).		(81) Designated States: AT (European patent), AU, BE (Euro- pean patent), CH (European patent), DE (European pa- tent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (Eu- ropean patent), IT (European patent), JP, KR, LU (Eu- ropean patent), NL (European patent), NO, SE (Euro- pean patent), SU. Published <i>With international search report.</i>
(54) Title: VIRUS FREE PLATELET DERIVATIVES AND METHOD OF MAKING SAME (57) Abstract A virus free composition containing active PDWHF and a method of making the same which has been heat treated to inactivate virus activity while maintaining activity of one or more PDWHF components. A further modified procedure in accordance with the present invention involves removing bacteria from a composition containing PDWHF by sterile filtration.		

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Title: VIRUS FREE PLATELET DERIVATIVES AND METHOD OF MAKING SAME

BACKGROUND OF THE INVENTION

1. Field Of The Invention

5 The present invention relates generally to virus free platelet derivatives and a method of making the same. More particularly, the invention relates to a composition comprising at least one platelet derived wound healing factor in which viral and bacterial activity has been deactivated or eliminated, while maintaining the biological activity of
10 such platelet derived wound healing factor, and a method of making the same.

2. Description Of The Prior Art

 It is vital to insure, to the extent possible, that any drug, including those derived from human or animal blood, is free of potential pathogens
15 such as viruses or bacteria. As used herein, the term drug is intended to mean any composition or product used in the medical treatment of animals or humans. For drugs which are synthetically prepared, this is generally not a major problem since the viral or bacterial activity can be controlled and thus eliminated during the manufacturing process. This is
20 not the case, however, with respect to drugs derived from biological sources whose desired activity is based upon the maintenance of such activity in the biological material. Biologically derived drugs are susceptible to carrying viruses, bacteria and other contaminants of the donor which, if not eliminated or deactivated, can be transmitted to the

recipient during treatment. This is a particularly acute concern in the case of human blood or drugs derived from human blood which can carry various infectious viruses such as Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV), etc. In recent years, with the
5 identification of HIV or the AIDS virus, total absence of HIV or the AIDS virus in human blood or blood derived drugs has become absolutely mandatory.

Two general approaches have been taken to insure virus free blood or blood derived drugs. One approach has involved treating the blood,
10 blood plasma, etc. with a sterilization or deactivation process following removal from a donor and prior to transfusion or preparation of the blood derived drug. Various procedures have been tested for the purpose of inactivating viruses in biological materials such as human blood. Some have had limited success, others have not. Procedures which have been
15 tested include, among others, neutralization of specific viruses with corresponding antibodies, ultraviolet irradiation, beta-propiolactone radiation, treatment with various detergents and solvents, etc. In general, these and other virus inactivation procedures have suffered from one or more deficiencies. In some cases, the inactivation is limited to a specific
20 virus, while in others the inactivation is incomplete or leads to the inactivation of the desired blood component activity as well. In still others, the procedure is unsafe or is laborious and time consuming and requires extensive incubation and other processing steps.

Application of heat has also been considered as a means to
25 inactivate viruses in blood plasma proteins such as immunoglobulins since it is known that heat applied at certain temperatures for certain periods of time will inactivate viral activity. Specifically, Patent No. 4,721,777 issued to Uemura et al. relates to a process for heat treating immunoglobulins in a dry state to inactivate viruses present therein.
30 Although Uemura et al. appears to have had some success in deactivating viruses in some plasma proteins by heat in a dry state, it is generally accepted that, except for albumin, plasma proteins and other blood

components cannot withstand liquid heating. These other blood components are commonly considered to have biological activities which are sensitive to heat and highly susceptible to thermal denaturation.

Because of the many problems involved in removing or
5 inactivating virus activity in blood and blood derived products, except for serum albumin which can withstand liquid heating, the general practice has been to take no step to inactivate viruses in blood supply. Accordingly, recipients of blood transfusions or blood derived drugs necessarily take the risk that the blood source is contaminated with HBV
10 or other infectious viruses. This risk has become particularly acute with the identification of HIV or the AIDS virus.

A second approach for insuring virus free blood or blood derived drugs, or at least minimizing the risk to recipients, has involved prescreening blood for specific viruses prior to its use. Prescreening
15 techniques have improved in recent years primarily in response to, and as a result of the ability to identify, the HIV or AIDS virus. Presently, prescreening is the generally accepted method of providing a safe blood supply. However, problems still exist with prescreening techniques. One problem, of course, is that it is difficult to screen for all viruses possibly
20 present. Instead, screening is conducted only for certain viruses of specific concern such as HIV or HBV. Secondly, erroneous results still can and do arise through prescreening techniques because of faulty readings or because HIV, HBV or other viruses are present in a form which has not progressed to a stage where it can be detected. Thus risks continue to exist
25 with respect to prescreening techniques as well.

Accordingly, there is a continuing need for virus free blood derivatives such as platelet derived wound healing formula and a method of preparing the same. There is also a need for a method of inactivating or removing various pathogens such as viruses and bacteria, and particularly
30 viruses, in such blood derivatives such as platelet derived wound healing formula which is safe and which does not adversely alter the desired activity of the derivative itself.

SUMMARY OF THE INVENTION

It is recognized in the prior art that the liquid heating of whole blood or blood components to inactivate viruses contained therein can cause protein denaturation and loss of biological activity. In fact, it is contemplated that heat treatment of blood at temperatures and for periods of time sufficient to inactivate HBV, HIV and other viruses contained therein would destroy the blood cell structure to the point where it would be unusable for any further processing such as the isolation and recovery of plasma derived platelets or platelet derived wound healing formula (PDWHF). In accordance with the present invention, however, it was unexpectedly discovered that if PDWHF which had previously been isolated and recovered is heat treated at temperatures and for incubation times sufficient to inactivate HBV and HIV, the wound healing activity of one or more wound healing components or factors of PDWHF is substantially retained. Such a result is unexpected and contrary to what would have been anticipated in view of the prior art.

The present invention also includes a method step which, in addition to inactivating viral contaminants via a heat treatment step, removes bacteria via a filtration step. Thus, a further feature of the present invention is to provide a composition comprising PDWHF which is free of any viral or bacterial activity while maintaining desired biological activity. Such composition is prepared by applying both a heat treatment and a filtration step to a composition containing PDWHF.

More specifically, the process of the present invention and the process by which the composition of the present invention is manufactured includes preparing a composition comprising PDWHF which contains at least one platelet derived wound healing component or factor. This can be accomplished by several processes known in the art. The preferred process is by removing platelets from platelet rich plasma via centrifugation, stimulating the release of PDWHF from the platelets by

the addition of thrombin or other activating agent and removing the spent platelets by centrifugation. This leaves a supernatant containing PDWHF. A second step in the process is to incubate the composition containing PDWHF for a time period and at a temperature sufficient to
5 inactivate HBV, HIV or any other desired virus. Preferably, in order to insure inactivation of HBV and HIV, the composition containing PDWHF is incubated at 60 C. for 10 hours.

A further step of a modified process in accordance with the present invention is to dilute the heat treated composition containing PDWHF to
10 a desired concentration and subject the same to a low protein binding membrane for filtration. Preferably, to remove certain undesirable bacteria, a filter having a pore size of 0.2 micrometers (or smaller) is desired.

Application of the preferred method of the present invention
15 results in a composition containing PDWHF in which various contagious viruses have been safely deactivated while maintaining biological activity of one or more of the individual wound healing or factors. It is contemplated that the present invention can be used either in lieu of or in addition to current blood prescreening
20 techniques.

Accordingly, it is an object of the present invention to provide a virus free composition comprising PDWHF while maintaining biological activity with respect to one or more individual wound healing components or factors.

25 Another object of the present invention is to provide a process for preparing a composition comprising active PDWHF which is free of contaminating viruses.

Another object of the present invention is to provide a composition comprising active PDWHF which is free of viral and bacterial activity and
30 a method of making the same.

A still further object of the present invention is to provide a method of removing or inactivating viral or bacterial activity in a

composition comprising PDWHF either in lieu of or in addition to a blood prescreening procedure.

These and other objects of the present invention will become apparent with reference to the detailed description of the invention and the appended claims.

DESCRIPTION OF THE FIGURES AND TABLES

Figure 1 is a set of three graphs reflecting the results of a fibroblast mitogenic assay on three different preparations of homologous PDWHF which shows the effect of heat on the respective homologous PDWHF preparations in comparison to a control.

Figure 2 is a set of three graphs reflecting the results of a fibroblast mitogenic assay for three different preparations of autologous PUWHF which shows the effect of heat on the respective autologous PDWHF preparations in comparison to a control.

Figure 3 is a set of three graphs reflecting the results of a PDWHF stimulated endothelial cell chemotaxis assay on three different preparations of homologous PDWHF which shows the effect of heat on the relative cell migration of the respective homologous PDWHF preparations in comparison to a control.

Figure 4 is a set of three graphs reflecting the results of a PDWHF stimulated endothelial cell chemotaxis assay on three different preparations of autologous PDWHF which shows the effect of heat on the relative cell migration of the respective autologous PDWHF preparations in comparison to a control.

Figure 5 is a graph reflecting the effect of different filter membranes on homologous PDWHF fibroblast mitogenic activity.

Figure 6 is a set of two graphs reflecting the results of a PDWHF stimulated endothelial cell chemotaxis assay for a Milipore "Durapore" filter for two different preparations of homologous PDWHF.

Figure 7 is a set of two graphs reflecting the results of a PDWHF stimulated endothelial cell chemotaxis assay for an S&S OE66 filter for two different preparations of homologous PDWHF.

Table I reflects the effect of heat on homologous and autologous PDWHF levels of β thromboglobulin (β TG) and platelet factor 4 (PF4).

Table II reflects the effect of different filter membranes used for sterile filtration on homologous PDWHF levels of β TG, and PF4.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, platelet derived wound healing formula (PDWHF) or a composition comprising PDWHF is provided which is free of viral and bacterial activity, while still maintaining desired biological activity of one or more wound healing components or factors of PDWHF.

Platelet derived wound healing formula (PDWHF) is a blood derivative involved generally in the wound healing process. It has previously been discovered that by artificially recovering PDWHF and applying the same to a wound, the healing process is stimulated and speeded. PDWHF, as that designation is used herein, comprises a mix of various individual components or factors which are involved in various roles and degrees in the wound healing process. At present, over fifty different components are known to exist in PDWHF. Among those individual platelet derived wound healing components or factors which are known or suspected to be involved in wound healing include the following, among others:

1. β Thromboglobulin (β TG) a chemotactic for fibroblasts which are required for formation of granulation tissue. β TG has the same sequence as CTAP III (below) minus the first four amino acids.
2. Connective Tissue Activating Protein III (CTAP III): MW

= 9273 Da single chain, pI 8.5. CTAP III is a mitogen for synovial fibroblasts.

3. Platelet Factor 4 (PF4) is a 7,772 Da polypeptide found as a tetramer of 30 kDa. It is released from platelets in a complex of two proteoglycan molecules and four PF4 tetramers with a combined MW of 350 kDa. PF4 is a chemotactic for neutrophils and monocytes which aid in wound debridement and combating infection.

4. Platelet Derived Growth Factor (PDGF) a mitogen for cells of mesenchymal origin including fibroblasts, smooth muscle cells and glial cells. It is also chemotactic for fibroblasts and smooth muscle cells. It is a 31-32 kDa dimer composed of either one or both polypeptide chains referred to as A and B. All three types of dimers are released from platelets i.e. AA, BB and AB.

5. Platelet Derived Angiogenesis Factor (PDAF) is approximately 7 kDa peptide. It is chemotactic for endothelial cells (but not mitogenic).

6. Platelet Derived Epidermal Growth Factor (PDEGF) is mitogenic for many types of cells and can stimulate epidermal growth and keratinization. Multiple forms have been observed from 6k to 149 kDa.

7. Platelet-Derived Endothelial Cell Growth Factor (PDECGF) is a 45 kDa protein which is mitogenic and chemotactic for endothelial cells.

8. Transforming Growth Factor beta (TGF β) is a pleiotropic factor which acts as a potent chemotactic for monocytes and subsequent release of additional healing factors, as well as stimulating other cells to differentiate secrete extracellular matrix materials, etc. TGF β is a 25 kDa dimer that is released from platelets in a 220-235 kDa latent form.

9. Adenosine Diphosphate (ADP, MW = 427 Da) has been shown to potentiate the action of other growth factors.
10. Plasminogen Activator Inhibitor (PAI) is a 47 kDa protein and is involved in activation of TGFb.
- 5 11. PDWHF may also contain Transforming Growth Factor alpha (TGFa, MW=5.7 kDa), basic Fibroblast Growth Factor (bFGF, MW=16.47 kDa, pI=9.6) and acidic Fibroblast Growth Factor (aFGF, MW=15.6 kDa, pI=5.6-6.0).

PDWHF is derived or released from blood platelets as a result of activation with thrombin or other activators including physical activation. The platelets in turn are derived from a whole blood source. The blood source can be autologous (from the same individual), homologous (from the same species) or heterologous (from different species). A process step preparatory to the virus inactivation step of the present invention is the preparation of the PDWHF product on which virus inactivation is to be performed. The desired PDWHF can be prepared using techniques known in the art and described in copending application Serial No. 039,776 filed April 15, 1987 and Serial No. 408,058 filed September 15, 1989, the disclosures of which are incorporated herein by reference.

Following treatment by thrombin or another activating agent, the spent platelets are removed from the released PDWHF by centrifugation. The resulting supernatant contains PDWHF including one or more of the platelet derived components or factors involved in wound healing. The PDWHF prepared in accordance with the above procedure can be stored until ready to proceed with the following virus inactivation step. It should be noted that the preparation of PDWHF or a composition containing PDWHF is known in the art and that the above is but one method of preparation.

The principal virus inactivation step in accordance with the present invention involves the heat treatment of PDWHF or composition containing one or more wound healing factors of PDWHF. As generally

known in the art, heat treatment of blood can result in protein denaturation and loss of biological activity. In fact, except for a process involving pasteurization of serum albumin, application of heat to blood or blood products at temperatures and for incubation periods sufficient to inactivate viruses is generally considered to be destructive of the desired biological activity of the blood or blood products. However, in accordance with the present invention, it was found that individual platelet derived wound healing factors of PDWHF unexpectedly retained their desirable activity when incubated at 60°C for up to 10 hours to inactivate viruses such as HBV, HIV, etc.

Although incubation for a period of 10 hours at 60°C is generally recognized as being sufficient to inactivate HBV and HIV, it is contemplated that PDWHF or compositions containing PDWHF can be heat treated at other temperatures and for shorter or longer incubation periods depending upon the particular virus or types of viruses whose inactivation is desired. Accordingly, the principal process step of the present invention involves the heat treatment of PDWHF or a composition comprising at least one wound healing factor of PDWHF at a temperature and for an incubation period sufficient to inactivate a specific virus or set of viruses while maintaining the biological activity of such wound healing factor at acceptable levels.

Generally, temperatures below about 30°C will have limited virus deactivation capability regardless of the incubation period, while temperatures above about 80°C, even for relatively short incubation periods, will result in unnecessary reduction of desired PDWHF component activity without significantly increasing the virus inactivation. The appropriate incubation time will depend primarily on the heat treatment temperature being utilized, the particular viruses whose deactivation is desired and the particular PDWHF wound healing factor whose activity maintenance is desired. In general, however, incubation times should be at least about 15 minutes and no more than about 24 hours. It is generally accepted, however, that incubation for 10

hours at a temperature of at least 60°C is sufficient to inactivate all possible viruses of concern including HBV and HIV.

It has been shown through various heat treatment studies that liquid heat treatment of PDWHF at a temperature and for an incubation period sufficient to inactivate viruses does not inactivate certain components or factors of PDWHF involved in wound healing. In these studies, wound healing activity of the PDWHF and various individual wound healing factors was confirmed by fibroblast mitogenic assays to determine maintenance of the mitogenic or cell dividing activity, endothelial chemotaxis assays to determine maintenance of chemotactic or cell migration activity, and enzyme-linked immunoassays (EIA) to determine the maintenance of the levels of certain individual factors, namely, PF4 and BTG. The details of these heat treatment studies are reflected in Figures 1-4 and Table I and are described as follows.

EXAMPLE 1

Heat Treatment Studies

Prior to conducting the various activity assays identified below, test quantities of three different homologous PDWHF samples (from apheresis packs) and three different autologous PDWHF samples (from whole blood) were prepared. Aliquots (0.5 ml each) of each homologous and autologous preparation sufficient to conduct the assays below and were incubated in a liquid state for 10 hours at 60°C. Similar aliquots of the homologous and autologous PDWHF preparations without heat treatment were used as controls. The biological wound healing activity of both the control and the heat treated preparations were then evaluated by subjecting the same to the following assays.

ASSAY 1(A)

Fibroblast Mitogenic Assay

The principal purpose of the fibroblast mitogenic assay was to ascertain the mitogenicity, or cell division capability, of PDWHF. The assays were done on several dilutions of the sample and the results expressed in counts per minute (cpm) of tritiated thymidine for various dilution levels and reflected in the graphs of Figures 1 and 2. The graphs of Figure 1 reflect the assay results conducted on the three homologous PDWHF preparations and controls, while the graphs of Figure 2 reflect the assay results conducted on the three autologous PDWHF preparations and controls. As shown, the results of the assays reflected in Figures 1 and 2 show no significant loss of fibroblast mitogenic activity in homologous or autologous PDWHF preparations which were heat treated at 60°C for up to 10 hours.

ASSAY 1(B)

Endothelial Cell Chemotaxis Assays

Each of the homologous and autologous PDWHF preparations and controls were also evaluated via an endothelial cell chemotaxis assay to determine the relative cell migration of the various preparations for various dilution factors. The results are reflected in the graphs of Figure 3 for the homologous PDWHF preparations and in the graphs of Figure 4 for the autologous PDWHF preparations. It is shown that although the heat treated PDWHF preparations (both homologous and autologous) exhibit a slightly altered response curve, the findings reflected by Figures 1-4 indicates that PDWHF and the wound healing factors thereof which are responsible for cell division and cell migration are still active.

ASSAY 1(C)

Enzyme-linked Immunoassays

EIA's were used to determine the concentration of the wound healing factors β TG and PF4 in the various preparations homologous and autologous PDWHF and controls. The results, except for autologous

PDWHF I, are identified in Table I. As shown in Table I, heat treatment of the preparations at 60°C for 10 hours did not cause any significant effect on the levels of β TG and PF4.

In addition to inactivating viruses in PDWHF via heat treatment, it is desirable to inactivate or remove contaminating bacteria as well. This is accomplished in accordance with the present invention by sterile filtration. It is contemplated, however, that the virus inactivating heat treatment may be utilized with or without bacterial filtration.

In accordance with the modified procedure of the present invention, sterile filtration is used to eliminate and remove bacterial and other larger pathogens. It is desirable and preferable, however, for the filter membrane to be a low protein binding membrane to prevent loss of PDWHF components during the filtering step. Use of high protein binding filter membranes generally result in substantial loss of certain PDWHF components and thus their associated biological activities. Filtration studies were conducted on the same preparations of homologous and autologous PDWHF used in the heat treatment studies. The purpose of the filtration studies was to evaluate the extent of retained biological activity following filtration through the following five membranes, each having pore size of 0.22 μ m:

<u>Filter No.</u>	<u>Description</u>
1	Millipore Corp., Bedford, MA hydrophilic "Durapore": polyvinylidene difluoride GVWP 02500
2	Schleicher & Schuell Inc. (S&S), Keene, NH OE66: cellulose acetate membrane 23600
3	Nuclepore Corp., Pleasanton, CA "Membra-Fil": mixed-esters of cellulose 140628
4	Gelman, Ann Arbor, MI "Supor": modified polysulfone 60300

5 Micron Separations, Inc.
 "Magna" nylon 66 membrane
 Fisher NO2-SPO24-00

 The detailed results and findings of the filter studies are
5 reflected in Figures 5, 6 and 7 and Table II and are described below.

EXAMPLE 2

Filtration Studies

 For the filtration studies, homologous PDWHF preparations
controls were evaluated. During these filtration studies, the above
10 identified five filter membranes were tested. All membranes tested were
25 mm (3.9 cm²), 0.22 micrometers pore size and were advertized as low
protein binding filters. Each membrane was supported in a 25 mm filter
holder. Using a syringe, 5 mls. aliquots of appropriately diluted samples of
each PDWHF preparation was passed through the filter, and then
15 compared to the unfiltered control material via the following assays.

ASSAY 2(A)

Fibroblast Mitogenic Assay

 Fibroblast mitogenic assays to evaluate mitogenic or cell
division activity were conducted for homologous PDWHF I with respect
20 to each of the above identified five filters. The results are reflected in
Figure 5 which plots tritiated thymidine in counts per minute (CPM)
against dilution of the material passed through each of the five
membranes tested and the controls. From these results, it can be seen that
fibroblast mitogenic activity was essentially eliminated by Filter No. 4 and
25 was substantially reduced by Filter No. 5. No significant loss of fibroblast
mitogenic activity was shown, however, with respect to Filter Nos. 1, 2
and 3. It is believed that these results suggest that even though Filter Nos.
4 and 5 are advertised as low protein binding filters, they tend to bind the

PDWHF components responsible for fibroblast mitogenic activity significantly more than the other filters tested.

ASSAY 2(B)

Endothelial Cell Chemotaxis Assay

5 Two of the above identified filters, namely Filter Nos. 1 and 2, were evaluated for maintenance of cell migration activity via an endothelial cell chemotaxis assay. Samples were then separately passed through Filter Nos. 1 and 2. Following the filtering step, serial dilution of the sample and controls were compared for cell migration activity. The
10 results for Filter No. 1 are reflected in Figure 6, while the results for Filter No. 2 are reflected in Figure 7. In both cases, little difference was observed between the control and Filter Nos. 1 and 2. The other Filter Nos. 3, 4 and 5 were not assayed.

ASSAY 2(C)

Enzyme-linked Immunoassays

15 Various enzyme-linked immunoassays were conducted with respect to two of the homologous PDWHF preparations. Such assays were conducted to determine the levels of β TG and PF4 in the filtrates from the above identified filters and to compare the same to the unfiltered controls.
20 EIA evaluations for both β TG and PF4 in homologous PDWHF I were conducted with respect to all five filters, while EIA evaluations for homologous PDWHF II were conducted only with respect to the control and Filter Nos. 1, 2 and 3. The results are reflected in Table II. As shown, substantial amounts of β TG and PF4 were lost when the PDWHF was
25 passed through Filter Nos. 4 and 5. Values obtained with Filter Nos. 1, 2 and 3, however, were substantially the same (given experimental error), as the unfiltered control.

Although it is contemplated that various modifications could be made to the invention as described in detail above, without deviating
30 from the spirit of the present invention, it is contemplated that the scope

of the present invention be dictated by the appended claims rather than by the detailed description set forth above.

CLAIMS

1. A method of inactivating selected viruses in a composition comprising at least one platelet derived wound healing factor comprising:
heating the composition at a temperature and for an
5 incubation period sufficient to inactivate said selected viruses without inactivating said one factor.
2. The method of claim 1 wherein said temperature is between about 30°C and 80°C.
3. The method of claim 1 wherein said temperature is at least 60°C.
- 10 4. The method of claim 1 wherein said incubation period is between about 15 minutes and 24 hours.
5. The method of claim 1 wherein said incubation period is at least about 10 hours.
6. The method of claim 1 wherein said temperature is between about
15 30°C and 80°C and said incubation period is between about 15 minutes and 24 hours.
7. The method of claim 6 wherein said temperature is about 60°C and said incubation period is about 10 hours.
8. The method of claim 1 wherein said heating step comprises a liquid
20 heating step.
9. The method of claim 1 wherein said one platelet derived wound healing factor contributes to the mitogenicity of said composition.
10. The method of claim 1 wherein said one platelet derived wound healing factor contributes to the relative cell migration capability of said
25 composition.
11. The method of claim 1 including removing contaminating bacteria by sterile filtration.
12. The method of claim 11 wherein said filtration is conducted via a low protein binding filter having a pore size sufficiently small to remove
30 said bacteria, but sufficiently large to pass said one factor.

13. The method of claim 12 having a pore size of less than about one micrometer and greater than about 0.01 micrometers.
14. The method of claim 12 wherein said filter has sufficiently low protein binding characteristics to preclude said one factor from binding to said filter.
15. The method of claim 1 wherein said composition comprises PDWHF.
16. The method of claim 1 wherein said one factor is β TG or PF4.
17. A composition comprising at least one platelet derived wound healing factor in which viral activity of selected viruses has been deactivated by heat treatment of said composition at a temperature and for an incubation period sufficient to inactivate said selected viruses without deactivating said one factor.
18. The composition of claim 17 wherein said composition is heat treated at a temperature of between about 30°C and 80°C.
19. The composition of claim 17 wherein said composition is heat treated at an incubation period of between about 15 minutes and 24 hours.
20. The composition of claim 17 where said composition is heat treated at a temperature of at least 60°C.
21. The composition of claim 20 wherein said composition is heat treated for an incubation period of at least 10 hours.
22. The composition of claim 21 wherein said composition is heat treated at a temperature of about 60°C for an incubation period of about 10 hours.
23. The composition of claim 17 being substantially free of contaminating bacteria.
24. The composition of claim 17 wherein said bacteria has been removed by sterile filtration.
25. The composition of claim 24 wherein said sterile filtration is conducted via a low protein binding filter having a pore size sufficiently small to substantially remove contaminating bacteria, but large enough to pass said one platelet derived wound healing factor.

26. The composition of claim 25 wherein said filter has a pore size of less than about one micrometer and greater than about 0.01 micrometers.

27. The composition of claim 25 wherein said filter has sufficiently low protein binding characteristics to preclude said one platelet derived wound healing factor from binding to said filter.

28. The composition of claim 17 comprising PDWHF having said one platelet derived wound healing factor.

29. The composition of claim 17 wherein said one factor is BTG or PF4.

30. The composition of claim 17 wherein said one factor contributes to the mitogenicity of said composition.

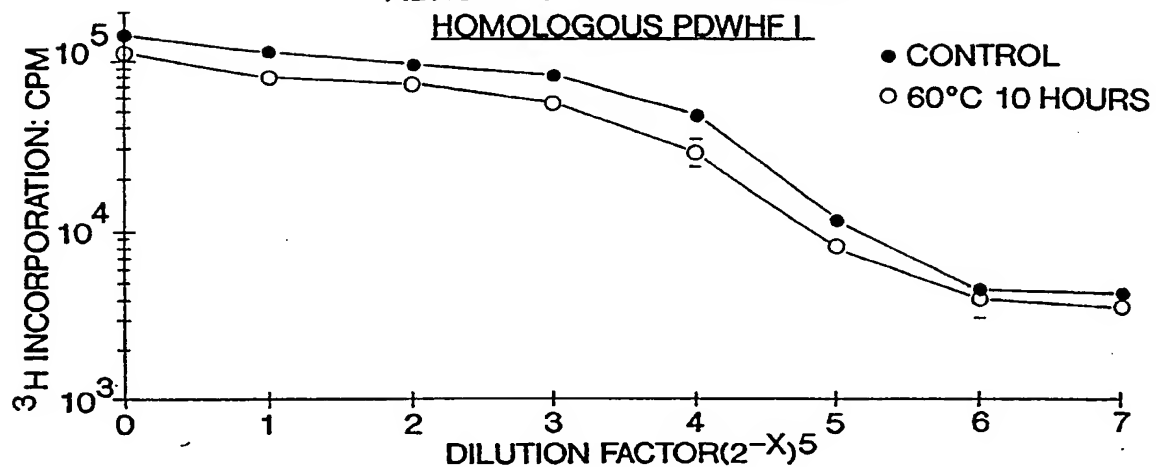
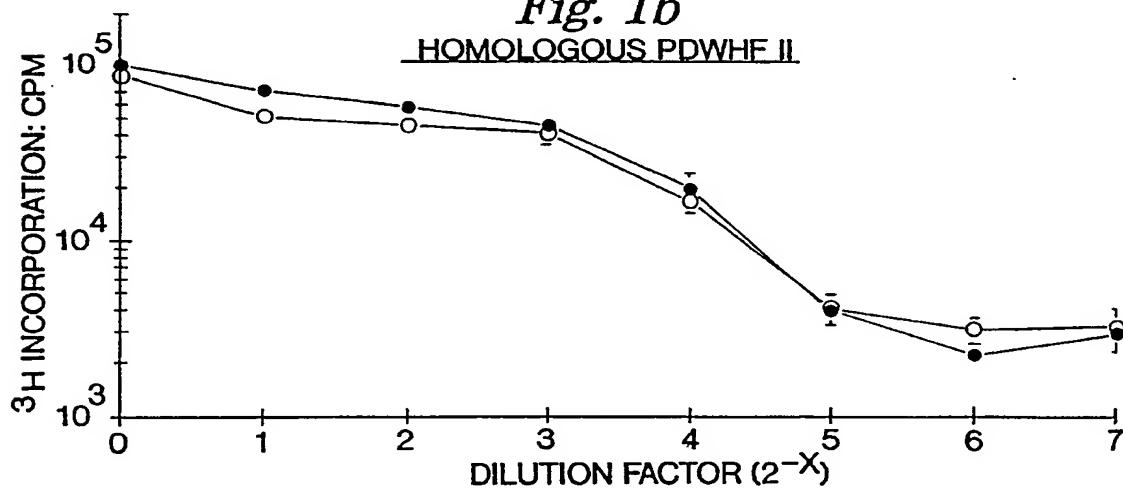
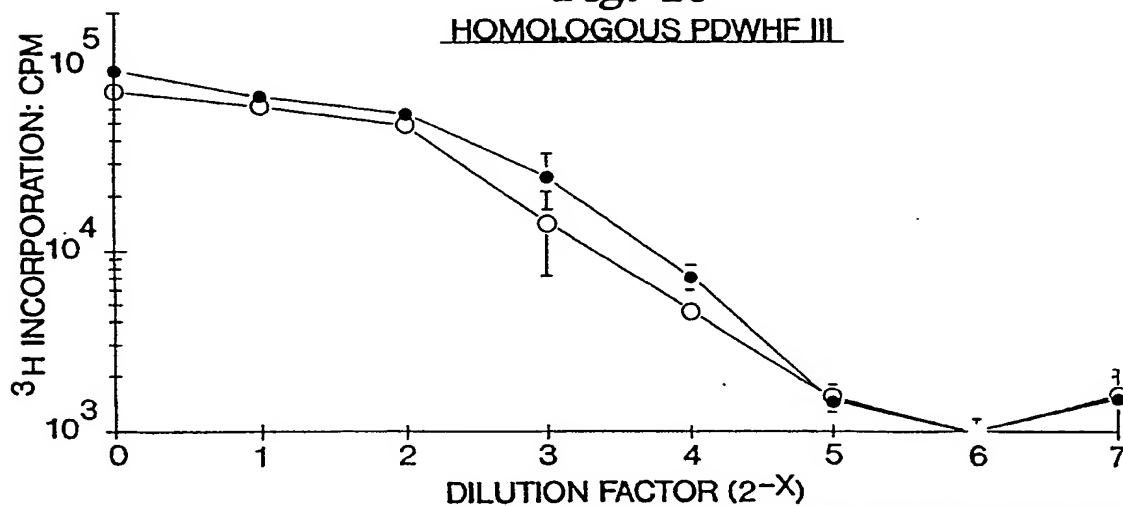
31. The composition of claim 17 wherein said one factor contributes to the relative cell migration capability of said composition.

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Fig. 1a

ASSAY 1(A)

FIBROBLAST MITOGENIC ASSAY

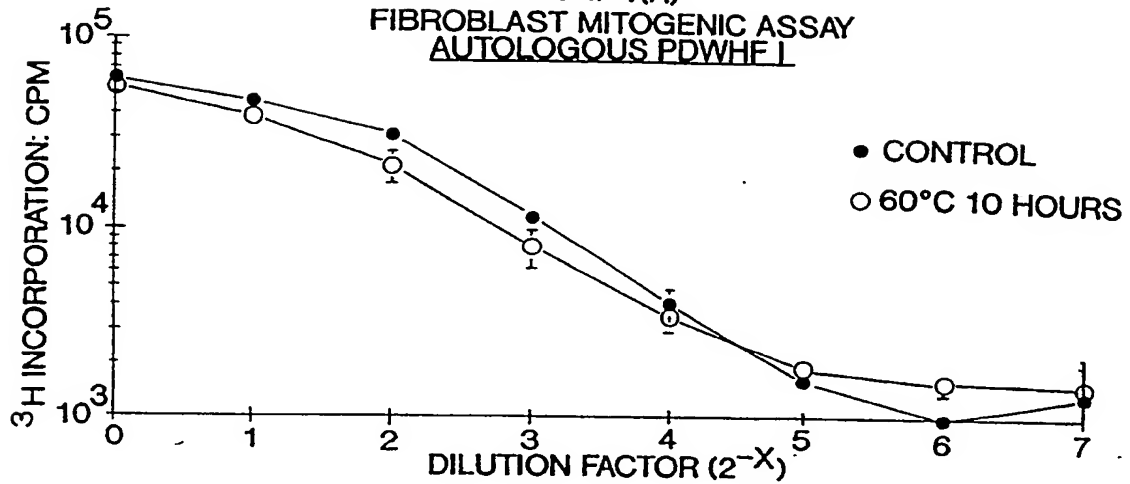
HOMOLOGOUS PDWHE I*Fig. 1b*HOMOLOGOUS PDWHE II*Fig. 1c*HOMOLOGOUS PDWHE III

SUBSTITUTE SHEET

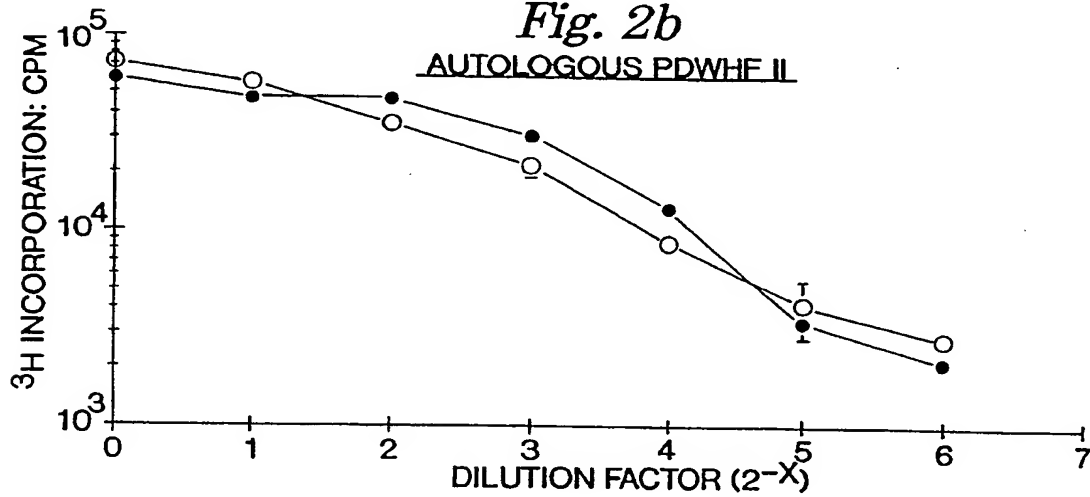
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Fig. 2a

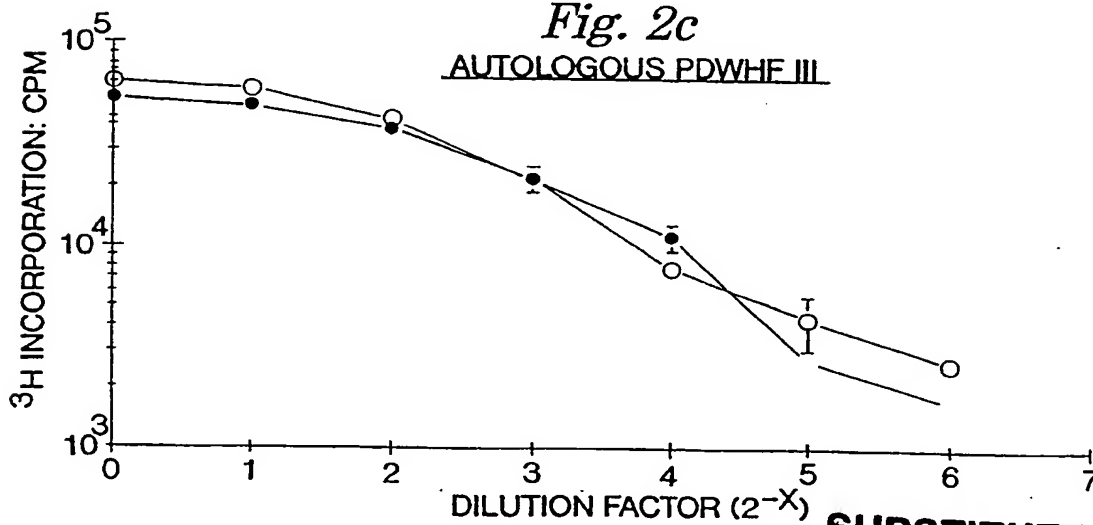
ASSAY 1(A)

FIBROBLAST MITOGENIC ASSAY
AUTOLOGOUS PDWHE I*Fig. 2b*

AUTOLOGOUS PDWHE II

*Fig. 2c*

AUTOLOGOUS PDWHE III



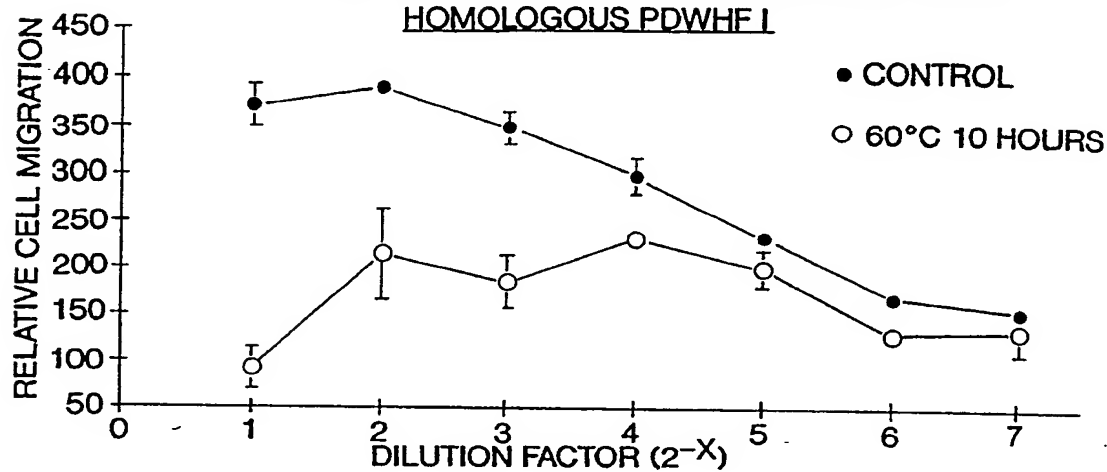
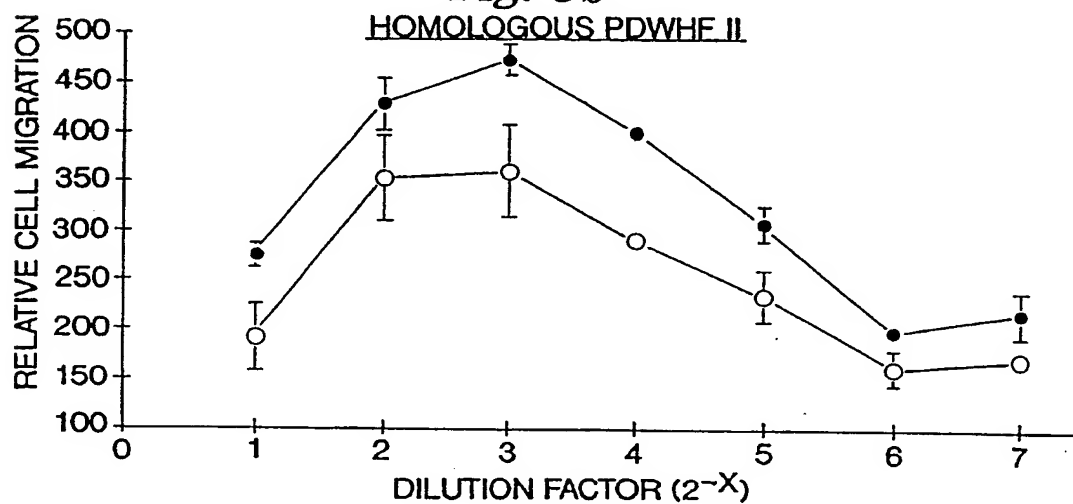
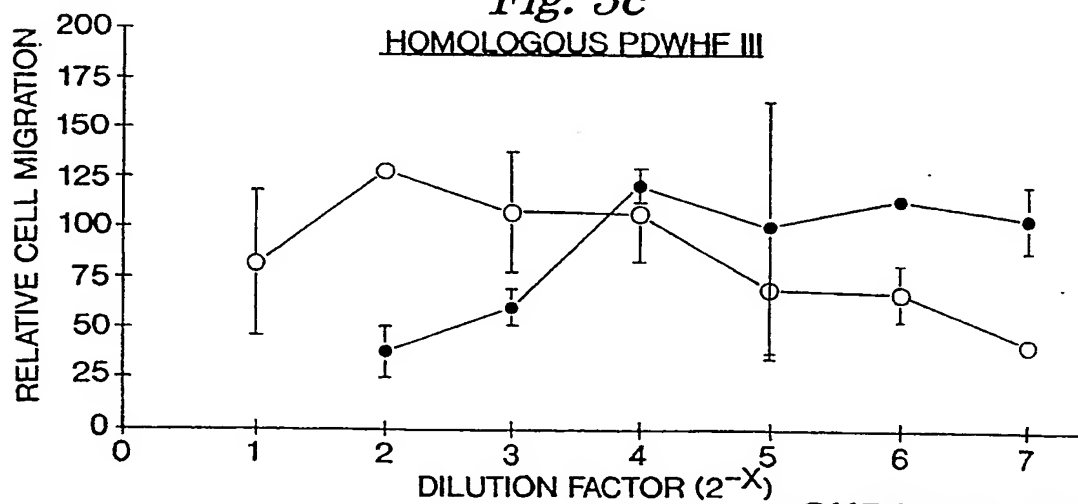
SUBSTITUTE SHEET

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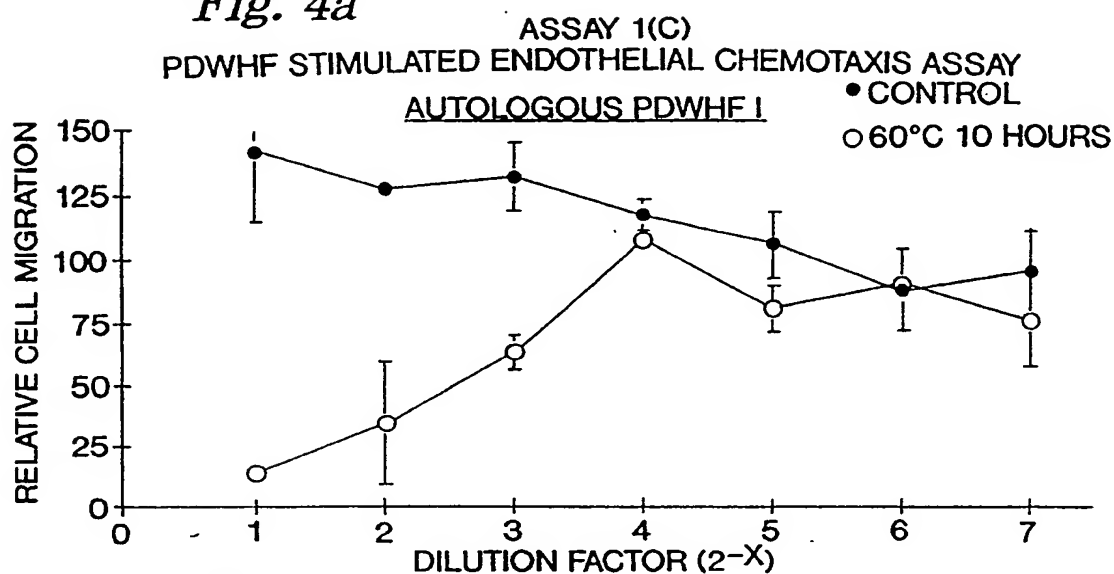
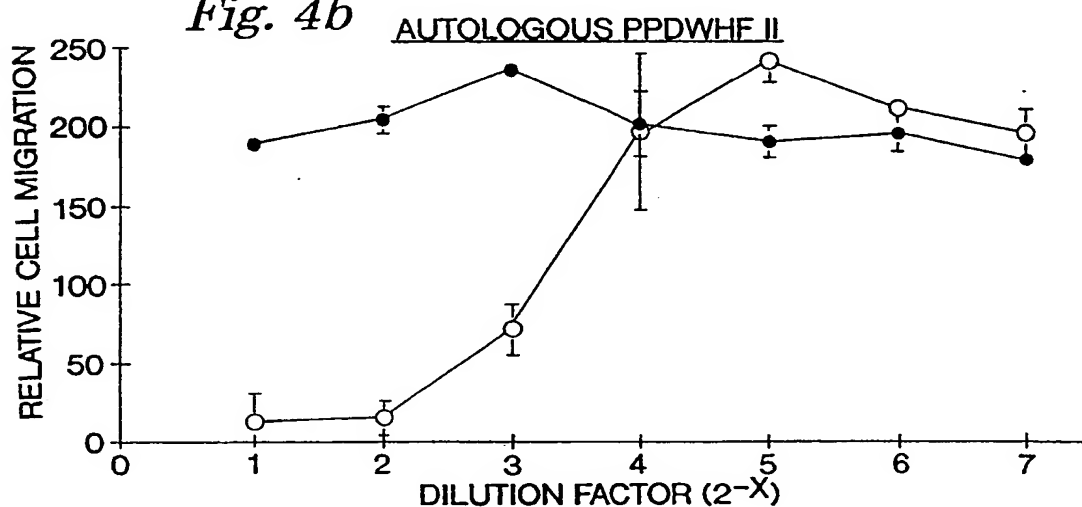
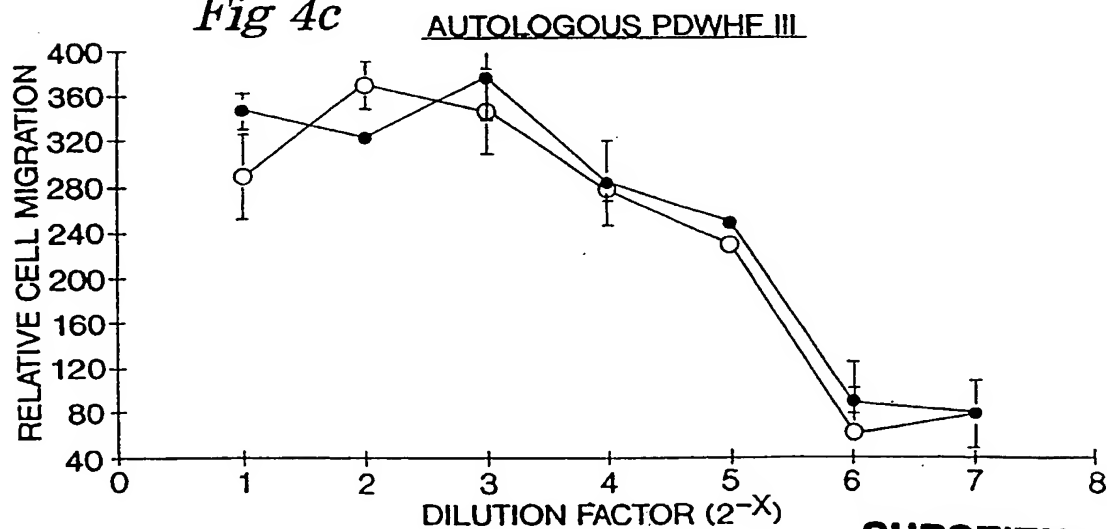
Fig. 3a

ASSAY 1(C)

PDWHF STIMULATED ENDOTHELIAL CHEMOTAXIS ASSAY

HOMOLOGOUS PDWHF I*Fig. 3b*HOMOLOGOUS PDWHF II*Fig. 3c*HOMOLOGOUS PDWHF III**SUBSTITUTE SHEET**

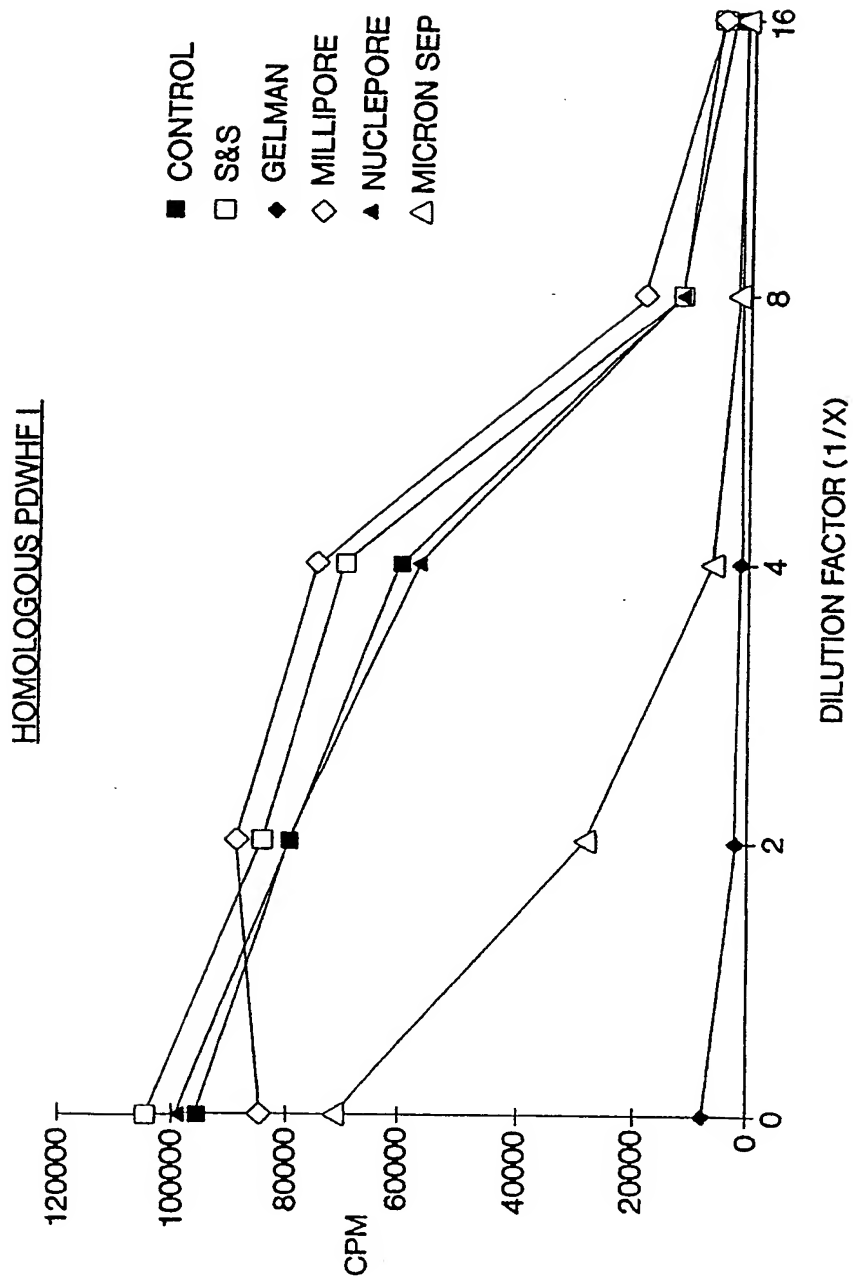
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Fig. 4a*Fig. 4b**Fig 4c*

SUBSTITUTE SHEET

Fig. 5

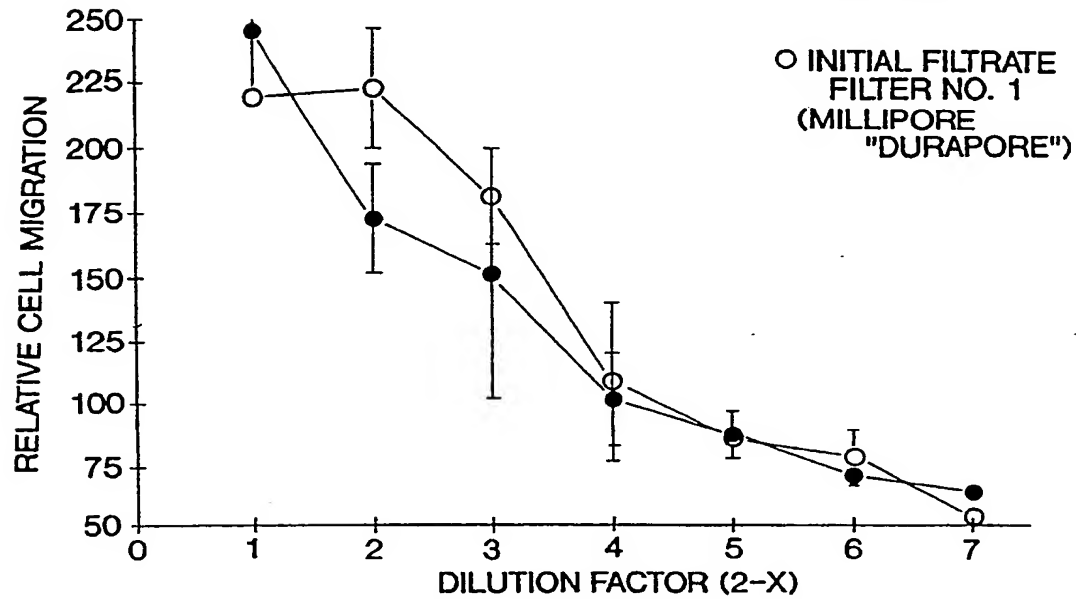
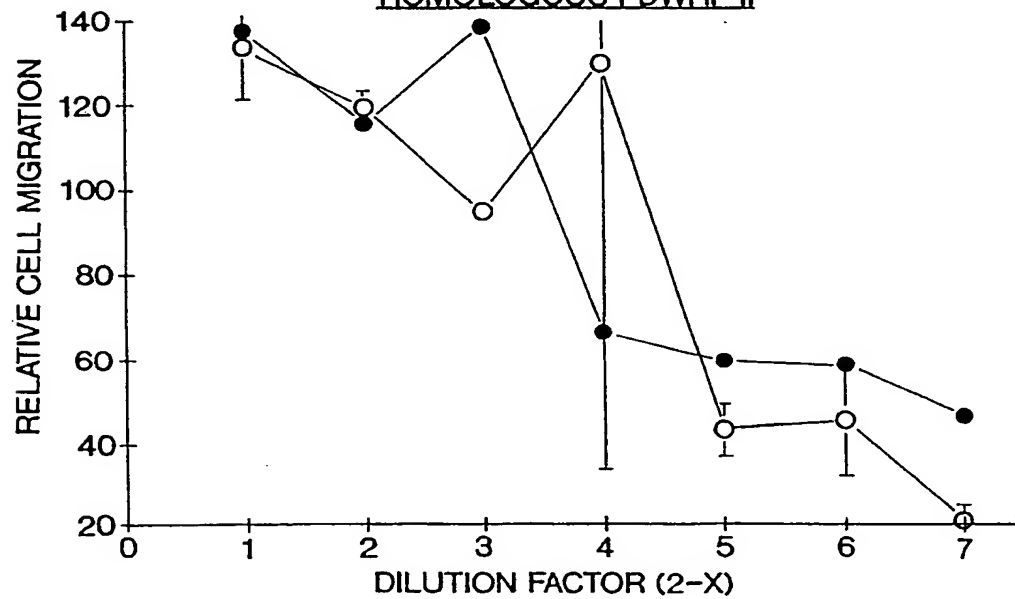
ASSAY 2(A)
FIBROBLAST MITOGENIC ASSAY



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*Fig. 6a*ASSAY 2(B)
PDWHF STIMULATED ENDOTHELIAL CHEMOTAXISHOMOLOGOUS PDWHF I

● CONTROL

○ INITIAL FILTRATE
FILTER NO. 1
(MILLIPORE
"DURAPORE")*Fig. 6b*HOMOLOGOUS PDWHF II

SUBSTITUTE SHEET

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Fig. 7a

PDWHF STIMULATED ENDOTHELIAL CHEMOTAXIS

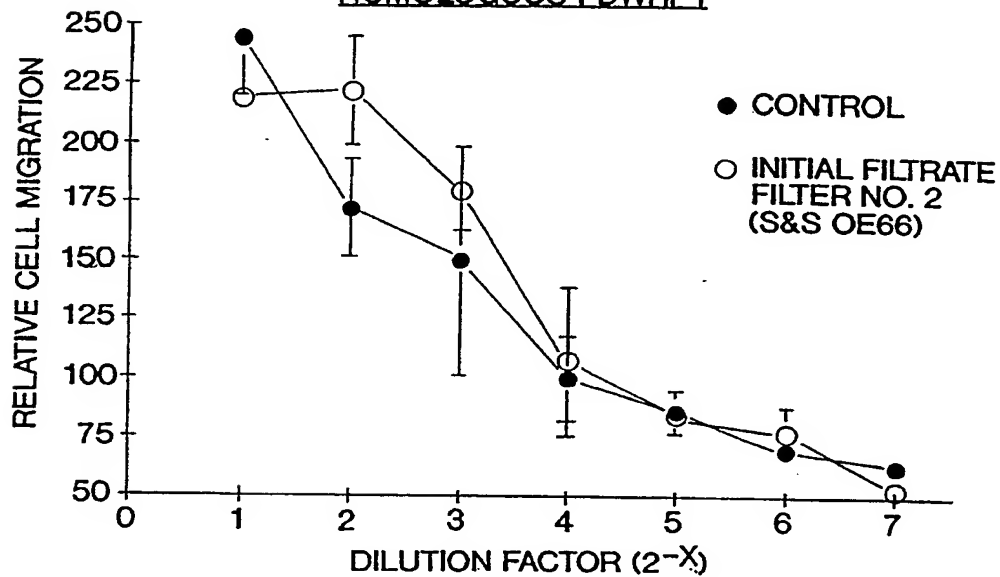
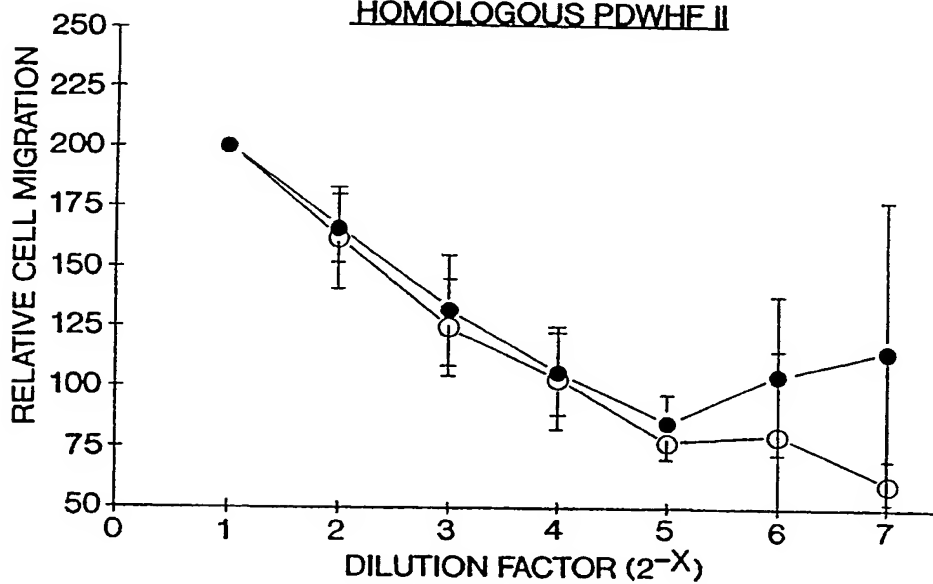
HOMOLOGOUS PDWHF I*Fig. 7b*HOMOLOGOUS PDWHF II

Table 1

	HOMOLOGOUS PDWHF			AUTOLOGOUS PDWHF		
	β THROMBOGLOBULIN PLATELET FACTOR 4	g/ml	%	β THROMBOGLOBULIN PLATELET FACTOR 4	g/ml	%
I CONTROL HEAT TREATMENT	22	100%	6.3	100%	5.8	100%
	22	100%	7.7	122%	5.2	90%
II CONTROL HEAT TREATMENT	26	100%	7.4	100%	18	100%
	26	100%	7.1	96%	21	117%
III CONTROL HEAT TREATMENT	16	100%	7.2	100%	16	100%
	17	106%	5.9	82%	18	113%

Table 2

	HOMOLOGOUS PDWHF I			HOMOLOGOUS PDWHF II		
	β THROMBOGLOBULIN PLATELET FACTOR 4	g/ml	%	β THROMBOGLOBULIN PLATELET FACTOR 4	g/ml	%
CONTROL (UNFILTERED)	10	100%	2.6	100%	7.0	100%
MILLIPORE: "DURAPORE"	10	100%	1.6	62%	7.1	101%
S&S OE66	10	100%	2.1	81%	6.2	89%
NUCLEPORE: "MEMBRA-FIL"	10	100%	3.2	123%	5.9	84%
MICRON: "MAGAN"	7.2	72%	0.6	23%	1.7	85%
GELMAN: "SUPOR"	0.5	5%	0	0%		

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/01545

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶

According to International Patent Classification (IPC) or to both National Classification and IPC
 IPC(5): C07K 3/12, 3/26, 3/28; A61K 37/36
 U.S.CL.: 530/344, 350, 380, 386, 399, 412, 414, 427; 514/2, 12, 21

II. FIELDS SEARCHED

Minimum Documentation Searched ⁷	
Classification System	Classification Symbols
U.S.CL.	530/344, 350, 380, 386, 399, 412, 414, 427; 514/2, 12, 21
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸	

III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹

Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	US, A, 4,350,687 (LIPTON ET AL) 21	1-27
Y	September 1982, see columns 4 and 8	1-27
Y	Journal of Interferon Research, Vol. 1 No. 1, issued 1980, Oleszak et al., "Platelet Derived growth Factor (PDGF) Inhibits Antiviral and Anticellular Action of Interferon in Synchronized Mouse or Human Cells", pages 37-48. See pages 38-39.	1-10, 15, 16 17-23, 28-31
A	US, A, 4,424,206 (Ohmura et al.) 03 January 1984. See entire document.	1-27
A	US, A, 4,479,896 (Antoniades) 30 October 1984. See entire document.	1-27
A	US, A, 4,721,777 (Uemura et al) 26 January 1988. See entire document.	1-27

^{*} Special categories of cited documents: ¹⁰

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

22 May 1991

International Searching Authority

ISA/US

Date of Mailing of this International Search Report

02 JUL 1991

Signature of Authorized Officer

Andrew G. Rozycki

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Experimental Cell Research, Vol. 135 No. 1, issued 1981, Dicker et al "Similarities Between Fibroblast Derived Growth Factor and Platelet- Derived Growth Factor". pages 221-227. See page 222.	1-27

